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AMYLOLYTIC AND CELLULOLYTIC ACTIVITIES, THE DIGESTIBILITY OF DRY MATTER OF BROILER CHICKENS AFTER FEED INTAKE OF HUMIC SUBSTANCES

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ABSTRACT

This study aimed to investigate the effects of feed intake of humic substances (HS) on the cellulolytic and amylolytic activities, and the digestibility of dry matter (DM) in the chymus of the intestine and ileum of broiler chickens (Cobb 500). Four groups of birds (A, B, C, negative control; n = 120) were fed with mash diets (starter HYD1 230.20, grower HYD2 222.20, finisher HYD3 209.40 g crude protein (CP) per kg of DM) with the added HS from day 1 to 37 days as follows: A – 0.7 % Humac nature; B – 0.7 % Humac nature monogastric (HNM); C – 0.5 % HNM (Humac Ltd., Slovakia). The cellulolytic activities were increased in the intestine in B/C by 2.50 (P < 0.01)/1.65 (P < 0.05) on day 3 and 1.02 (P < 0.001)/0.3 (P < 0.05) µmol.l^{-1}.min^{-1}.g^{-1} on day 24. There were observed higher values of digestibility coefficient of DM in the intestine (P < 0.05) in C by 15.14 % and 14.85 %, and in B by 20.73 % on days 17, 24 and 31, respectively. The enhancement of ileal digestibility of DM was observed in B by 7.38 % (P < 0.05) and CP in A and B by 3.7% and 6.1% (P < 0.05). The feed intake of HS favourably influenced the cellulolytic activities and increased the DM digestibility in the intestine and the ileum, as well as the CP digestibility in the ileum.

Key words: crude protein; dry matter, humates; intestinal digestibility; poultry nutrition

INTRODUCTION

The breeding of broiler chickens is a very important sector of poultry farming which provides animal-based proteins. The importance of broiler chicken meat production stands out and poultry meat is the best alternative to red meat. It is a source of high-quality protein, minerals, and vitamins which are used to balance the human diet [24].

Various kinds of feed additives and their combinations are utilized for the improvement of production and health parameters of poultry such as: probiotics and prebiotics [3, 12], organic acids [23], enzymes [35] and plant extracts [2, 27, 28]. In addition to the feed supplements, humic substances (HS) have been attracting the attention of scientists for several years.

The humic substances (HS) are components of the organic part of the soil, geological organic deposits and soil water. Their presence was demonstrated in lake sediments, brown coals and peats. From the chemical point of view, HS are heterogenous mixtures of polydispersed compounds produced as a result of chemical reactions in the

time of transformation of plants or organic matter during humification. The substrates used in the process of humification are: polysaccharides, proteins, nucleic acids, lipids, melanin, plant lignin, cutin and char particles [19]. HS are considered as supramolecular associations of self-organizing heterogenous and relatively small molecules which resulted from the decomposition of plant residues [33].

The characteristics of HS are based of the solubility at various pH and retention on hydrophobic resins. Three main components are: humic, fulvic acids (FA) and humin (HA). Whereas, humic acids are soluble at higher pH and insoluble at pH < 2, fulvic acids are soluble under all pH values and humin is insoluble in acids and alkalis [31].

The application of HS in animal breeding is bound with its adsorptive, anti-inflammatory and antibacterial activities [25]. Many experiments testing their positive characteristics have been performed with animal species like pigs [34, 36], sheep [40], cattle [42] and laboratory micromammals [41]. Several significant positive results have been achieved in poultry farming with broiler chickens and laying hens [6, 22].

However, different results of the feed conversion ratio and digestibility of broiler chickens have been published. In the case of production parameters, the effects of 0.7 % supplementation of HS on the growth performance of broiler chickens were not so significant [18], as on the composition and quality of breast meat. On the contrary, broilers fed with HS extracted from worm compost in the drinking water, showed lower feed conversion ratio (FCR), higher energy digestibility and higher retention of dry matter (DM), ash, N and energy [15].

A scientific hypothesis was based on the positive influence of HS on the enzymatic activities in the gastrointestinal apparatus of poultry with the effect on the digestion of dry matter in the intestine.

The aim of this study was to investigate the effects of dietary intake of HS on the amylolytic and cellulolytic activities in the intestine as well as on the digestibility of DM in broiler chickens.

MATERIALS AND METHODS

Broiler chickens and feed

One-day-old broiler chickens of the hybrid Cobb 500 with an average weight of 50 g were supplied by a commercial hatchery. The chickens were randomly divided into 3 experimental groups and a control group of 30 birds. They were housed in floor pens in a shed specialized for poultry production. The area for one broiler chicken was 0.12 m^2. Access to feed and water was constant.

Starter (HYD1), grower (HYD2) and finisher (HYD3) ground feeds were fed for 37 days. Starter and grower diets contained an anticoccidial agent (salinomycin sodium), while all other diets did not contain antimicrobial agents.

HS were added to the feeds of the experimental groups (A, B, C) as components of two products. The first HN was added to the feed of group A in the amount of 0.7 %, the second HNM was added to the feeds of groups B or C in the amounts of 0.7 % and 0.5 %, respectively.

The HN or HNM products had the following parameters: Particle size up to 100 mm, maximum moisture content of 15 % and the content of

- HA min. 650/570, FA min. 50/50 $g.kg^{-1}$,
- Ca 42.28/51.1, Mg 5.11/4.86, Fe 19.05/18.09 $g.kg^{-1}$,
- Cu 15/14.25, Zn 37/35.15, Mn 142/135,
- Co 1.24/1.18, Se 1.67/1.59 and
- Mo 2.7/2.57, V 42.1/40 $mg.kg^{-1}$ of DM.

The calculated content of HA/FA in feeds used in the experimental groups was the following: A 4.55/0.35, B 3.99/0.35, C 2.85/0.25 $mg.kg^{-1}$. The measurement of zootechnical performance was performed once a week.

Ethical statement

Experiments were performed under the European Directive for the protection of vertebrate animals used for experimental and other scientific purposes (86/609/EU) and with the permission of the State Veterinary and Food Administration of the Slovak Republic No. 3090/13-221 in the poultry breeding facilities of the University of Veterinary Medicine and Pharmacy in Košice, Slovakia.

Analysis of feed

The analysis of the experimental feeds was performed according to the methods (Table 1) of the Association of Official Analytical Chemists (AOAC) [8].

The determination of dry matter (DM), crude protein (CP), crude fat (CF), starch, and ash was performed. Neutral detergent and acid detergent fibres were analysed using the methods by Van Soest et al. [39]. The atomic absorption spectrophotometry was used for the determination of the mineral composition of the feeds [38]. The

Table 1. Nutrients and ingredients in feeds

Nutrients [g.kg^{-1}]	Feed mixtures		
	Starter HYD1	Grower HYD2	Finisher HYD3
Dry mater	1000.00	1000.00	1000.00
Crude protein	230.20	222.20	209.40
Crude fat	31.30	83.80	67.70
NDF	112.60	122.10	128.80
ADF	54.60	62.50	68.10
Ash	57.30	60.60	52.10
Ash insoluble in HCl	2.10	1.80	2.40
Starch	485.60	446.80	448.60
Calcium	4.93	6.00	7.41
Phosphorus	5.73	7.93	5.13
Sodium	2.98	1.93	1.60
Magnesium	2.86	3.06	3.11
Potassium	9.03	8.83	8.49
Copper	0.0275	0.0578	0.0594
Zinc	0.0229	0.0294	0.1336
Manganese	0.0789	0.1472	0.1437
Metabolisable energy [MJ.kg^{-1}]*	13.27	14.30	13.58
Ingredients			
HYD1/HYD2/HYD3	maize, wheat, soybean meal, vegetable oil, limestone, amino acids and their salts, monocalcium phosphate, lysine, methionine, mineral-vitamin premix		
HYD1/HYD2	anticoccidial agent salinomycin sodium 70 mg.kg^{-1}		

* – [13]; NDF – neutral detergent fibre; ADF – acid detergent fibre

phosphorus was quantitated spectrophotometrically [7]. The insoluble portion of ash in HCl in the feed mixture was determined [9]. The metabolisable energy (ME) was calculated using a formula from Commission Regulation (EC) No. 152/2009 [13], which described the method of calculation and expression of energy value.

Enzymatic activities and intestinal digestibility

Both kinds of analyses were performed at 17, 24 and 31 days of age. All samples of excreta were taken directly from the cloaca into sterile glass containers.

Samples for quantification of digestive enzyme activities were prepared as follows: The fresh sample (1 g) was diluted with 49 ml of sterile TBS buffer (TRIS-hydroxymethyl aminomethane 10 mmol.l^{-1}, HCl 0.5 mol.l^{-1}, pH 7.0) and homogenised. The diluted samples were then used for the determination of the amylolytic and cellulolytic activities [26] which were analysed with the substrates starch (Fisher Slovakia Ltd.) and methyl hydroxyethylcellulose (Merck Ltd., Germany). The DM, ash and percent of insoluble ash in HCl were determined by the methods mentioned in the feed analysis. The calculation of the digestibility coefficient of the DM of chicken was done according to the method of G u g l i e m o and K a r a s o v [16], modified by M a r c i n et al. [29].

Measurement of ileal digestibility

Analysis of ileal digestibility was performed after slaughter on day 37. The samples of ileal chyme were collected in sterile glass containers. For the determination of DM, ash and ash insoluble in HCl, the above methods were used.

The calculation of the ileal digestibility was performed according to J o h n s o n et al. [21]. The comparison of nutrients in the feed in the ileal chyme referred to the content of the insoluble part of the ash and was used to determine digestibility according to the following formula:

Ileal digestibility (%) = (A/B – C/D) * B/A * 100

where,

A – nutrient content in the diet [g.kg^{-1}], B – indicator in the diet [g.kg^{-1}], C – nutrient output in the ileum [g.kg^{-1}], and D – indicator in the ileum [g kg^{-1}]

The insoluble portions of ash in HCl in feeds and in the ileal chymus were used as the indicators in the diets and ileum [9].

Statistical analysis

The data were statistically analysed using IBM SPSS Statistics, version 24. The results of treatments were compared by one-way analysis of variance and by the Tukey-Kramer multiple comparison test.

RESULTS

Zootechnical performance of broiler chickens

The use of HS as a feed additive had a positive effect on improving the performance and nutrient digestibility of poultry [11].

Table 2. Growth data of broiler chickens

Groups (n = 30)	BW [g] in days							ADWG [g.day^{-1}]
	1	7	14	21	28	35	37	1–37
A	50.0 ± 3.75	182.07^{b} ± 4.66	488.97 ± 65.44	963.07 ± 120.89	1571.48 ± 280.39	2311.92 ± 367.72	2506.67^{b} ± 160.47	66.39 ± 16.85
B	50.0 ± 2.65	188.82^{ab} ± 5.85	512.75 ± 67.64	986.37 ± 116.20	1636.63 ± 210.66	2326.56 ± 292.20	2490.25^{b} ± 166.39	65.95 ± 17.77
C	50.0 ± 3.35	192.27^{ab} ± 7.18	521.5 ± 45.87	989.33 ± 102.5	1585.17 ± 286.5	2281.93 ± 326.51	2377.75^{ab} ± 133.19	62.91 ± 18.06
Control	**50.0 ± 4.15**	**191.73^{a} ± 8.41**	**514.86 ± 54.30**	**985.57 ± 100.03**	**1633.0 ± 193.26**	**2291.79 ± 320.88**	**2319.42^{a} ± 92.55**	**61.34 ± 17.31**

Means (mean ± SD) with different superscript letters differ significantly ^{ab} – P < 0.05; ^{ac} – P < 0.01;
BW – body weight; ADWG – average daily weight gain

Table 3. Average daily feed intake and feed conversion ratio of broiler chickens

Groups (n = 30)	ADFI [g.day^{-1} per chicken] in days						FCR [kg. kg^{-1}]
	7	**14**	**21**	**28**	**35**	**37**	**1 – 37**
A	28.11	60.39	105.82	147.09	167.20	145.18	1.65 ± 0.29
B	27.29	66.54	112.17	152.38	188.36	154.71	1.64 ± 0.19
C	29.11	60.42	100.95	135.24	177.83	149.52	1.53 ± 0.26
Control	**27.94**	**63.62**	**106.12**	**140.82**	**156.10**	**146.38**	**1.51 ± 0.17**

ADFI – average daily feed intake; FCR – feed conversion ratio

Growth data of broiler chickens are shown in Table 2. The addition of HS 0.7 % to the diet of groups A and B improved the value of the average daily weight gain (ADWG) by 8.23 % and 7.52 %, respectively, compared with the control on days 1–37. A significant decrease in individual BW was observed on day 7. The value was 9.66 g lower in the A group compared to the control group ($P < 0.05$). The values of BW at the end of the experiment were increased in A and B groups by 187.25 and 170.83 g ($P < 0.05$) on day 37.

The values of the average daily feed intake (ADFI) and feed conversion ratio (FCR) are shown in Table 3. Although the improvement of ADFI was observed in the experimental groups, no significant differences in FCR were determined.

Enzymatic activities in the intestinal apparatus

The feed additive containing HS had a favourable effect on the increase of the cellulolytic activities in the intestine (Table 4) of chickens in groups B or C by 2.50 ($P < 0.01$) and 1.65 ($P < 0.05$) $\mu mol.l^{-1}.min^{-1}.g^{-1}$ on day 3 as well as by 1.02 ($P < 0.001$) and 0.3 ($P < 0.05$) $\mu mol.l^{-1}.min^{-1}.g^{-1}$ on day 24. The only decrease of this parameter was in the A or B groups on day 17 ($P < 0.05$) by 1.77 and 1.99 $\mu mol.l^{-1}.min^{-1}.g^{-1}$, respectively. In the case of the amylolytic activity, there were not observed significant differences in the experimental groups compared to the control.

Digestibility of dry matter in the intestinal apparatus

The feed intake of HS had a beneficial effect on the digestibility coefficient of DM (Table 5) in the intestine

Table 4. Digestive enzyme activities in the intestinal chyme of broiler chickens

Age [day]	Groups (n = 6)	Amylolytic (glucose) [$\mu mol.l^{-1}.min^{-1}.g^{-1}$]	Cellulolytic (glucose) [$\mu mol.l^{-1}.min^{-1}.g^{-1}$]
3	A	2.72 ± 0.604	3.98^{a} ± 0.811
	B	6.28 ± 3.440	5.33^{c} ± 0.614
	C	4.26 ± 0.485	4.48^{b} ± 0.396
	Control	**3.85 ± 0.726**	**2.83^{a} ± 0.162**
10	A	3.14 ± 0.706	3.08 ± 0.554
	B	2.59 ± 0.213	3.10 ± 0.172
	C	2.93 ± 0.117	2.52 ± 1.436
	Control	**3.08 ± 0.520**	**2.63 ± 0.459**
17	A	0.87 ± 0.315	1.37^{b} ± 0.294
	B	1.65 ± 0.409	1.15^{b} ± 0.267
	C	1.10 ± 0.120	2.05^{a} ± 0.865
	Control	**1.05 ± 0.481**	**3.14^{a} ± 0.769**
24	A	0.37 ± 0.090	0.59^{b} ± 0.016
	B	0.76 ± 0.263	1.38^{d} ± 0.198
	C	0.46 ± 0.278	0.66^{b} ± 0.048
	Control	**0.55 ± 0.418**	**0.36^{a} ± 0.085**
31	A	2.26 ± 0.229	3.01 ± 1.627
	B	1.75 ± 0.533	2.99 ± 0.262
	C	1.29 ± 0.169	2.16 ± 0.401
	Control	**1.67 ± 0.390**	**1.75 ± 0.381**

Means (mean ± SD) with different superscript letters differed significantly: ^{a,b} – $P < 0.05$; ^{a,c} – $P < 0.01$; ^{a,d} – $P < 0.001$

Table 5. Digestibility coefficient of dry matter

Groups (n = 6)	Digestibility coefficient [%] in days		
	17	24	31
A	56.82[a] ± 6.057	56.83[a] ± 4.356	55.56[a] ± 8.891
B	54.59[a] ± 5.327	58.58[a] ± 4.501	71.29[b] ± 9.441
C	66.04[b] ± 2.700	61.14[b] ± 2.689	61.50[a] ± 7.715
Control	**50.90[a] ± 2.525**	**46.29[a] ± 8.368**	**50.56[a] ± 6.054**

DM – dry matter; HS – humic substances; means (mean ± SD) with different superscript letters differed significantly: [a, b] – $P < 0.05$

Table 6. The ileal digestibility of dry matter on day 37

Groups (n = 6)	DM [%]
A	33.27[a] ± 5.26
B	38.32[b] ± 0.18
C	23.66[c] ± 0.56
Control	**30.94[a] ± 2.41**

DM – dry matter; HS – humic substances; means (mean ± SD) with different superscript letters differed significantly: [a, b] – $P < 0.05$; [c] – $P < 0.01$

of the experimental group. Higher values were observed after intake of 0.5 % or 0.7 % HNM in feed in the C or B groups compared to control by 15.14 %, 14.85 % and 20.73 % ($P < 0.05$) on days 17, 24 and 31.

Ileal digestibility of DM was increased at the inclusion level of HA/FA at a minimum of 3.99/0.35 g.kg^{-1} feed (Table 6) on day 37. A higher value was observed in the B group by 7.38 %.

DISCUSSION

The results of the growth data of broiler chickens indicated that the dietary intake of HS was effective in the enhancement of the zootechnical performances of broilers. A similar beneficial effect on the growth performance in broiler chickens (final body weight, daily weight gains) was observed by Gomes-Rosales and deAngeles [14] when worm leachate containing HS was mixed at levels of 20 % to 30 % in the drinking water. The results of Jaďuttová et al. [20] confirmed our findings. They observed that the addition of 10 g.kg^{-1} feed of humic substances had a positive insignificant effect on the growth parameters, breast and thigh meat yield, improved blood parameters and Ca content in bones of broiler chickens (Cobb 500). On the contrary, Hassan et al. [17] added 5 or 10 g of humate per kg of feed for the broilers (1–28 days of age). They observed a negative effect of the higher dose of the additive on the productive performance as regards to feed conversion ratio and performance index. The possible explanation for the positive action of HS on the production parameters of chickens was provided by Arif et al. [1]. The HS formed a protective film on the mucus epithelia of the gastrointestinal apparatus against infectious pathogenic agents and toxins which resulted in an increased weight gain and final weight of the animals.

The results of the average daily feed intake and the feed conversion ratio of broiler chickens are consistent with the findings reported by Bahadori et al. [5]. They demonstrated that the increase in the daily weight gains, the decrease in the feed intake and the decrease of the feed conversion ratio with the addition of the earthworm meal containing HS from 10–30 g.kg^{-1} DM. Similarly, Demeterová et al. [10] determined the improvement of the feed conversion ratio and the European efficiency index of broiler chickens on day 41 as the effect of the dietary addition of HS combined with the probiotic strain *Enterococcus faecium*. Jaďuttová et al. [20] indicated that feeding with HS at the level of 0.8 % and 1.0 % resulted in similar feed consumption in all groups but the improvement of the feed conversion ratio was especially in the group of broiler chickens with 1.0 % addition of HS. On the contrary, the growth performance and yield of breast and thighs did not show any difference between control and experimental groups of broiler chickens after feed intake of the extract of HS [11].

The explanation of the improvement of zootechnical performances can be based on the enhanced digestive enzymatic activities influenced by HS which are ensuring better utilisation of nutrients from feed. The second interpretation of the positive influence of HS on FCR, provided by Avci et al. [4] involves the fact that humic acids stabilise the intestinal microflora, therefore ensuring better utilisation of nutrients from the feed.

The data of the amylolytic and cellulolytic activities in the intestinal chymus of intestine of broilers are in

agreement with the experimental results achieved with sheep [30] when the peroral intake of HS increased the amylolytic and cellulolytic activities in the forestomach of sheep. The effect of HS on the amylolytic and cellulolytic activities can be influenced by the amount of the additive applied to the feed. T a k l i m i et al. [37] demonstrated that a feed additive based on HS can improve the digestion coefficient of nutrients as the effect of improved enzymatic activities, the increased gut length and the lessening in the passage rate of the intestinal contents.

The beneficial influence of HS on the digestibility coefficient of DM in the intestine and on the ileal digestibility of DM was confirmed in our experiments. The digestibility of nutrients present in the chymus of the intestine or ileum depends on the enzymatic activities in the intestinal apparatus which could be positively influenced by HS. G o m e z - R o s a l e s and d e A n g e l e s [14] observed the increased ileal digestibility of the quadratic response ($P < 0.05$) by increasing the dose of HA in the drinking water and the improved retention of nutrients. The possible explanation for the improved nutrient digestibility could be the increasing of villus length, then the increased area for absorption of nutrients after feed intake of HS [37]. On the other hand, HS have been reported to stabilize the intestinal microbiota when the increase in the counts of lactic acid bacteria and the decrease in enterobacteria in the intestinal content were observed [32]. It can have a positive effect on intestinal digestibility as well.

CONCLUSIONS

The feed intake of humic substances was beneficial in the enhancement of the cellulolytic activities in the intestine and positively influenced the intestinal and the ileal digestibility of DM, as well. The enhancement of the finishing body of birds resulted from the favourable physiological effects of HS in the intestinal apparatus.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

1. **Arif, M., Rehman, A., Abd El-Hack, M. E., Saeed, M., Khan, F., Akhtar, M., et al., 2018:** Growth, carcass traits, cecal microbial counts, and blood chemistry of meat-type quail fed diets supplemented with humic acid and black cumin seeds. Asian-Australas. *J. Anim. Sci.*, 31, 12, 1930–1938. DOI: 10.5713/ajas.18.0148.
2. **Arpášová, H., Pistová, V., Hrnčár, C., Fík, M., Haščík, P., 2018:** The impact of the humic acid and phytobiotics on performance and carcass parameters of broiler chickens. *Acta Fytotechn. Zootechn.*, 21, 4, 173–178. DOI: 10.15414/afz. 2018.21.04.173–1 78.
3. **Algawany, M., Abd El-Hack, M. E., Farag, M. R., Sachan, S., Karthik, K., Dhama, K., 2018:** The use of probiotics as eco-friendly alternatives for antibiotics in poultry nutrition. *Environ. Sci. Pollut. Res. Int.*, 25, 11, 10611–10618. DOI: 10.1007/s11356-018–1 687-x.
4. **Avci, M., Denek, N., Kaplan, O., 2007:** Effects of humic acid at different levels on growth performance, carcass yields and some biochemical parameters of quails. *J. Anim. Vet. Adv.*, 6, 1, 1–4. Available at https://medwelljournals.com/abstract/?doi=javaa. 2007.1.4. Accessed August 16, 2022.
5. **Bahadori, Z., Esmaielzadeh, L., Karimi-Torshizi, M. A., Seidavi, A., Olivares, J., Rojas, S., et al., 2017:** The effect of earthworm (*Eisenia foetida*) meal with vermi-humus on growth performance, haematology, immunity, intestinal microbiota, carcass characteristics, and meat quality of broiler chickens. *Livest. Sci.*, 202, 74–81. DOI: 10.1016/j.livsci. 2017.05.010.
6. **Bailey, C. A., White, K. E., Domke, S. L., 1996:** Evaluation of Menefee HumateTM on the performance of broilers. *Poult. Sci.*, 75, Suppl. 1, 84.
7. **Carvalho, L. H. M., De Koe, T., Tavares, P. B., 1998:** An improved molybdenum blue method for simultaneous determination of inorganic phosphate and arsenate. *Ecotoxicol. Environ. Restoration*, 1, 1, 13–19.

8. **Cunniff, P., 1995:** *Official Methods of Analysis of AOAC*. 16th edn., Association of Official Analytical Chemists, Arlington, USA.
9. **Daněk, P., Paseka, A., Smola, J., Ondráček, J., Bečková, R., Rozkot, M., 2005:** Influence of lecithin emulsifier on the utilisation of nutrients and growth of piglets after weaning. *Czech J. Anim. Sci.*, 50, 10, 459–465.
10. **Demeterová, M., Mariščáková, R., Pistl, J., Naď, P., Šamudovská, A., 2009:** The effect of the probiotic strain *Enterococcus faecium* DSM 7134 in combination with natural humic substances on performance and health of broiler chickens. *Berl. Munch. Ttierarztl. Wochenschr.*, 122, 9–10, 370–377.
11. **Dominguez-Negrete, A., Gómez-Rosales, S., de Lourdes Angeles, M., López-Hernández, L. H., Reis-de Souza, T. C., López-García, Y., et al., 2019:** Effect of the addition of humic substances as growth promoter in broiler chickens under two feeding regimens. *Animals*, 9, 12, 1101. DOI: 10.3390/ani9121101.
12. **El-Ghany, W. A., Abdel-Latif, M. A., Hosny, F., Alatfeehy, N. M., Noreldin, A. E., Quesnell. R. R., et al., 2022:** Comparative efficacy of postbiotic, probiotic, and antibiotic against necrotic enteritis in broiler chickens. *Poult. Sci.*, 101, 8, 101988. DOI: 10.1016/j.psj.2022.101988.
13. **European Commission, 2009:** Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *OJEU*, 54. 1–130. http://data.europa.eu/eli/reg/2009/152/oj. Accessed July 5, 2022.
14. **Gómez-Rosales, S., de Angeles, M., 2015:** Addition of a worm leachate as source of humic substances in the drinking water of broiler chickens. Asian-Australas. *J. Anim. Sci*., 28, 2, 215–222. DOI: 10.5713/ajas.14.0321.
15. **Gómez-Rosales, S., Angeles, M. L., López-Hernández, L. H., López-Garcia, Y. R., Domínguez-Negrete, A., 2022:** Responses of broiler chickens fed low or high non-starch polysaccharide diets and the addition of humic substances from a worm compost. *Braz. J. Poult. Sci.*, 24, 4, 11, 1087. DOI: 10.1590/1806-9061-2021–1 510.
16. **Gugliemo, C. G., Karasov, W. H., 1993:** Endogenous mass and energy losses in ruffed grouse. *The Auk*, 110, 2, 386–390.
17. **Hassan, S. M., 2014:** Effect of adding dietary humate on productive performance of broiler chicks. *Asian J. Poult. Sci.*, 8, 2, 23–31. DOI: 10.3923/ajpsaj.204.23.31.
18. **Hudák, M., Semjon, B., Marcinčáková, D., Bujňák, L., Naď, P., Koréneková, B., et al., 2021:** Effect of broilers chicken diet supplementation with natural and acidified humic substances on quality of produced breast meat. *Animals*, 11, 1087. DOI: 10.3390/ani11041087.
19. **International Humic Substances Society (IHSS), 2007:** What are humic substances? *Newsletter*, IHSS, Number 38, 2. http://humic-substances.org/wp-content/uploads/NL_38_2007.pdf. Accessed August 15, 2022.
20. **Jaďuttová, I., Marcinčáková, D., Bartkovský, M., Semjon, B., Harčárová, M., Nagyová, A., et al., 2019:** The effect of dietary humic substances on the fattening performance, carcass yield, blood biochemistry parameters and bone mineral profile of broiler chickens. *Acta Vet. Brno*, 88, 307–313. DOI: 10.2754/avb201988030307.
21. **Johnson, L. A., Deep, A., Classen, H., 2014:** Digestibility and performance responses of broiler chickens fed a pea-based diet with different levels of dietary microbial phytase. *USURJ*, 1, 1, 39–44. DOI: 10.32396/usurj.vlil.53.
22. **Kocabagli, N., Alp, M., Acar, N., Kahraman, R., 2002:** The effects of dietary humate supplementation on broiler growth and carcass yield. *Poult. Sci.*, 81, 227–230. DOI: 10.1093/ps/81.2.227.
23. **Konieczka, P., Józefiak, D., Kinsner, M., Smulikowska, S., 2021:** Effects of high-moisture corn preserved with organic acids combined with rapeseed meal and peas on performance and gut microbiota activity of broiler chickens. *Anim. Feed Sci. Technol.*, 280, 115063. DOI: 10.1016/j.anifeedsci.2021.115063.
24. **Kralik, G. Kralik, Z., Grčević, M., Hanžek, D., 2018:** Quality of chicken meat. In **Yücel, B., Taşkin, T.:** *Animal Husbandry and Nutrition*. 1st edn., Intechopen, 63–94. DOI: 10.5772/intechopen.72865.
25. **Kühnert, M., Bartels, K. P., Kröll, S., Lange, N., 1991:** Veterinary pharmaceuticals containing humic acid for therapy and prophylaxis for gastro-intestinal diseases of dog and cat. *Mon. Vet.*, 46, 1, 4–8.
26. **Lever, M., 1977:** Carbohydrate determination with 4-hydroxybenzoic acid hydrazide (PAHBAH): Effect of bismuth on the reaction. *Anal. Biochem.*, 81, 1, 21–27. DOI: 10.1016/0003-2697(77)90594-2.
27. **Levkut, M., Marcin, A., Lenhardt, Ľ., Porvaz, P., Revajová, V., Šoltysová, B., et al., 2010:** Effect of sage extract on alkaline phosphatase, enterocyte proliferative activity and growth performance in chickens. *Acta Vet. Brno*, 79, 2, 177–183. DOI: 10.2754/avb201079020177.
28. **Levkut, M., Marcin, A., Revajová, V., Lenhardt, Ľ., Danielovič, I., Hecl, J., et al., 2011:** Influence of oregano extract

on the intestine, some plasma parameters and growth performance in chickens. *Acta Vet.*, 61, 2–3, 215–225. DOI: 10.2298/AVB1103215L.

29. **Marcin, A., Levkut, M., Revajová, V., Šoltysová, B., Naď, P., 2016:** Influence of Salvia officinalis essential oil on digestion parameters and intestinal microflora of broiler chickens. *Folia Vet.*, 60, 1, 5–14. DOI: 10.1515/fv-2016-0001.
30. **Marcin, A., Bujňák, L., Mihok, T., Naď, P., 2020:** Effects of humic substances with urea on protozoal population and fermentation in the rumen of sheep. *Bulg. J. Vet. Med.*, 23, 1, 60–69. DOI: 10.15547/bjvm.2199.
31. **McCarthy, M. D., Bronk, D. A., 2008:** Analytical methods for the study of nitrogen. In C**apone, D. G., Bronk, D. A., Mulholland, M. R., Carpenter, E. J.:** *Nitrogen in the Marine Environment*. 2nd edn., Academic Press, Elsevier, 1219–1275. DOI: 10.1016/B978-0–1 2-372522-6.00028–1.
32. **Mudroňová, D., Karaffová, V., Pešulová, T., Koščová, J., Cingeľová Maruščáková, I., Bartkovský, M., et al., 2020:** The effect of humic substances on gut microbiota and immune response of broilers. *Food Agric. Immunol.*, 31, 1, 137–149. DOI: 10.1080/09540105.2019.1707780.
33. **Piccolo, A., 2002:** The supramolecular structure of humic substances. A novel understanding of humus chemistry and implications in soil science. *Adv. Agron.*, 75, 57–134. DOI: 10.1016/S0065-2113(02)75003-7.
34. **Písařiková, B., Zralý, Z, Herzig, I., 2010:** The effect of dietary sodium humate supplementation on nutrient digestibility in growing pigs. *Acta. Vet. Brno*, 79, 349–353. DOI: 10.2754/avb201079030349.
35. **Slominski, P., 2011:** Recent advances in research on enzymes for poultry diets. *Poult. Sci.*, 90, 9, 2013–2023. DOI: 10.3382/ps.2011-01372.
36. **Schuhmacher, A., Gropp, J. M., 2000:** Effect of humic acids on health state and performance of weaners. *Proc. Soc. Nutr. Physiol.*, 9, 77.
37. **Taklimi Seyed Mozafar, S. M., Ghahri, H., Isakan, M. A., 2012:** Influence of different levels of humic acid and esterified glucomannan on growth performance and intestinal morphology of broiler chickens. *Agric. Sci.*, 3, 5, 663–668. DOI: 10.4236/as.2012.3508.
38. **Van Loon, J. C., 1980:** *Analytical Atomic Absorption Spectroscopy, Selected Methods*. Academic Press, New York, 348 pp.
39. **Van Soest, P. J., Robertson, J. B., Lewis, B. A., 1991:** Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74, 10, 3583–3597. DOI: 10.3168/jds.S0022-0302(91)78551-2.
40. **Váradyová, Z., Kišidayová, S., Jalč., D., 2009:** Effect of humic acid on fermentation and ciliate protoyoan population in rumen fluid of sheep *in vitro*. *J. Sci. Food Agric*., 89, 1936–1940. DOI: 10.1002/jsfa.3675.
41. **Yasar, S., Gokcimen, A., Aluntas, I., Yonden, Z., Petekkaya, E., 2002:** Performance and ileal histomorphology of rats treated with humic acid preparations. *J. Anim. Physiol. Anim. Nutr. (Berl.)*, 86, 7–8, 257–264. DOI: 10.1046/j.1439-0396.2002.00383.x.
42. **Yüca, S., Gul, M., 2021:** Effect of adding humate to the ration of dairy cows on yield performance. *Ankara Univ. Vet. Fak. Derg.*, 68, 7–14. DOI: 10.33988/auvfd.626066.

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